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(54) Abstract Title
Wound treatment composition comprising insulin

(57) Insulin provides reliable and effective prevention of scarring and/or at least a reduction in the severity of scarring. The application of insulin to wounds topically or by local injection is particularly advantageous since it simultaneously reduces/prevents scarring whilst enhancing re-epithelialisation of the wound and thus provides a dual action wound healing treatment. The present invention accordingly provides a highly effective prophylactic treatment for any individual suffering tissue trauma to reduce and/or prevent normal and/or pathological scarring.

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WOUND TREATMENT COMPOSITION

This invention relates to the pharmaceutical use of compositions for application to wounds. In particular, the invention relates to the manufacture of a medicament for use in the treatment of scarring.

When injury, disease or surgery disrupts the normal architecture of body tissues such as the skin, eye and palmar aponeurosis of the hand, the body instigates a complex cascade of events collectively known as wound healing. These processes in the early human foetus lead to total regeneration of the damaged or destroyed tissue. However, in post natal humans, although the capacity of the outer layer of the skin, the epidermis, for regeneration is phenomenal, wound healing of the deeper skin layer, the dermis, is often accompanied by a fibroproliferative response that leads to the formation of a fibrotic scar. The tissues of the eye, particularly the cornea, often scar after surgery or trauma which reduces the vision of the individual. Dupuytren's disease affects the palmar aponeurosis of the hand. This condition is caused by scarring and fibrosis that causes contraction of the palmar structure, thus pulling the fingers permanently down towards the palm.

The severity of scarring of an individual in response to injury, disease or surgery is highly variable and depends on multiple factors, such as infection, wound extent and orientation. Nevertheless, even when all these factors are taken into account, the severity of so-called "normal" scarring still varies dramatically between individuals. This variation in the severity of scarring of the skin is perhaps most dramatically illustrated by the comparison of normal scars with

pathological scarring conditions such as hypertrophic scarring.

5 Hypertrophic scars are characterised by extensive scar
tissue, which contains an excess of collagen and is
highly cellular (Rockwell et al., 1989. Plast. Recon.
Surg. 84: 827 -837). Redness, hypopigmentation or
hyper-pigmentation of the affected area often
10 accompanies these scars. Patients can also suffer from
hyperaesthesia and pruritus and, in addition,
contraction of scars located over a joint can lead to a
loss of mobility. This distressing pathological
condition can affect substantial numbers of patients
who have suffered various types of skin trauma, with
15 children under the age of four years particularly
prone. Two of the latest estimates of the proportion of
paediatric burns patients who suffer from this
condition are as high as 44 and 60%.

20 At present there is no method of predicting which
individuals will develop these scars, nor any method of
preventing their formation or that of normal scars, nor
any treatment. Effective therapies for both
pathological scarring and normal scarring accordingly
25 continue to be sought.

It has been suggested for some time that insulin and
its related growth factor family, insulin-like Growth
Factors (hereinafter referred to as IGFs), may improve
30 the re-epithelialisation of wounds. US 5591709 and US
5461030 of Life Medical Sciences Inc describe topically
applied wound treatment formulations which are useful
for treating wounds by accelerating wound healing. The
formulations optionally contain insulin, together with
35 further specified components. Although many groups
(such as Pierre et al., 1998. J. Trauma. 44:34-345),

have shown that systemic treatment with insulin and IGFs increases the speed of wound closure, the affect of these agents on scar tissue formation has never before been investigated.

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To date, with regard to the development of anti-scarring therapies, attention has been focussed on the finding that transforming growth factor beta 1 (hereinafter referred to as TGF β 1) enhances scarring.

10

Many groups have worked to develop ways of inhibiting TGF β 1, with some success. Methods used have included TGF β 1 receptor blocking antibodies or the application of mannose-6-phosphate, which prevents the activation of latent TGF β 1. The main problem with these approaches is that induction of scarring is not the only function of TGF β 1. TGF β 1 is known for its angiogenic properties. In addition, application of low doses of TGF β 1 is known to enhance re-epitheliasation of wounds. Thus, although the blocking of either the action or activation of TGF β 1 in incisional wounds may result in the reduction of scarring with relatively few deleterious side effects, the healing of larger wounds, which rely on epithelial migration for closure, may be delayed.

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We have now surprisingly shown that insulin prevents and/or reduces the formation of scar tissue whilst also improving the speed of re-epithelialisation of the wound. It is effective in preventing/reducing scarring of all types of wounds such as incisional wounds and larger wounds.

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According to a first aspect of the present invention there is provided the use of insulin for the manufacture of a medicament for reducing and/or preventing scarring.

According to a second aspect of the present invention there is provided the use of insulin for the manufacture of a medicament for a dual action wound treatment comprising reducing and/or preventing
5 scarring whilst accelerating and/or promoting wound healing.

According to a third aspect of the present invention there is provided a method of reducing and/or
10 preventing scarring comprising administering a pharmaceutical composition comprising insulin to a wound.

We have now surprisingly found that insulin provides
15 reliable and effective prevention of scarring and/or at least a reduction in the severity of scarring. The application of insulin to wounds topically or by local injection is particularly advantageous since it simultaneously reduces/prevents scarring whilst
20 enhancing re-epithelialisation of the wound and thus provides a dual action wound healing treatment. The present invention accordingly provides a highly effective prophylactic and curative treatment for any individual suffering tissue trauma to reduce and/or
25 prevent normal and/or pathological scarring.

Insulin

Insulin is a small (mw~6000) polypeptide hormone
30 produced by the beta cells of the islets of Langerhans of the pancreas. It is made up of two chains of amino acids (designated A and B) which are held together by two disulphide bridges. Both chains are formed from the cleavage of a single helical chain known as pro-
35 insulin, which consists of both the A and B chains of insulin connected by a peptide termed the C-peptide.

Although the insulins of various species are highly homologous (differing only by a few amino acids) the sequence and number of amino acids making up the C-peptide can vary considerably. Under proper
5 conditions, three dimers of insulin associate to form a hexamer of appropriate dihedral symmetry that is stabilised by the presence of two zinc ions. Insulin affects cell metabolism via receptors present on the cell surface.

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The term "insulin" as used herein includes within its scope a wide variety of insulin forms, and mixtures thereof. Suitable insulins are commercially available
15 from Hoechst, Lilly, Novo Nordisk and CP Pharmaceuticals, for example.

Insulins that are suitable for the uses in accordance with the present invention can be sourced from a
20 variety of different species due to the high degree of homology of insulin between species. Preferred insulins are those that are commonly available, including porcine, bovine or human insulins or mixtures thereof. The human insulins tend to be either derived
25 by enzymatic modification and purification from porcine insulin or originate from microorganisms using standard recombinant DNA technology techniques. Insulins that are suitable for the uses in accordance with the present invention include conventional insulins,
30 single-peak insulins, highly purified insulins, monocomponent insulins, purified insulins, human insulin (emp), semisynthetic insulins, human insulin (crb), human insulin (prb), human insulin (pyr) and biosynthetic human insulin. A wide variety of insulin
35 forms are suitable for the uses of the present invention for example, crystalline insulin, soluble

insulin, neutral insulin, regular insulin and
unmodified insulin, and formulations that prolong the
duration of action of insulin such as suspensions
formed by complexing insulin with a protein from which
5 it is slowly released (examples are "protamine zinc
insulin" and "isophane insulin") or by modifying the
particle size (e.g., insulin zinc suspensions) or
biphasic insulins which are mixtures providing both
immediate and prolonged action.

10 Chemical modifications of the insulin molecule has
resulted in insulin such as delanated insulin (where
the C-terminal alanine has been removed from the B
chain of insulin), insulin defalan (where the terminal
15 phenylalanine has been removed), sulphated insulin,
insulin argine and insulin lispro. Such chemically
modified insulins are also suitable for the uses in
accordance with the present invention. Furthermore,
insulins obtained by standard recombinant DNA
20 technology are included within the scope of the present
invention. Insulins obtained by standard recombinant
DNA technology using nucleic acid chains that have a
sequence identical to the naturally occurring gene
encoding insulin in humans or other mammals are
25 preferred. This nucleic acid sequence may be modified
by either conservative base substitutions, such that it
encodes the same amino acid sequence of naturally
occurring insulin; or modified with base substitutions
which encode a different amino acid sequence from that
30 naturally occurring. Recombinant DNA technology has
enabled production of other insulin analogues with
altered pharmacokinetic profiles which are also
included within the scope of the present invention.

35 The insulin to be employed in the uses according to the
present invention is present in the pharmaceutical

composition in an effective amount. Normally the total amount of the active is present in an amount between 50 picograms (1.25×10^{-6} IU) to 1000 micrograms (25 IU) per millilitre of the composition. More preferably the amount is 5 nanograms (1.25×10^{-4} IU) to 500 micrograms (12.5 IU) and most preferably from 50 nanograms (1.25×10^{-3} IU) to 50 micrograms (1.25 IU), in order to maximise benefits at minimum cost.

10 Pharmaceutically Acceptable Vehicle

Most preferably the medicament in which the insulin is formulated for the uses according to the present invention is either a topical composition or an injectable composition for injecting locally i.e. at or near to the site of the wound to be treated.

Topical and/or injectable compositions used according to the present invention also comprise a pharmaceutically acceptable vehicle to act as a diluent, dispersant or carrier for the insulin. The vehicle may comprise materials commonly employed in wound treatment products such as water, saline solution, and, for topical compositions, liquid or solid emollients, silicone oils, emulsifiers, solvents, humectants, thickeners, powders, propellents and the like.

The vehicle will usually form from 0.1% to 99.9%, preferably 25% to 80% and can, in the absence of other adjuncts, form the balance of the composition.

In a particularly preferred embodiment the topical composition comprises a delivery polymer that is saturated with the appropriate concentration of insulin.

By "delivery polymers" is meant naturally occurring and/or synthetic polymers which facilitate the delivery of the active agent to its site of action. These polymers include hydrated or unhydrated hydrogels (e.g. hydroxyethylmethacrylate (HEMA), glycerolmethacrylate (GMA) and polyvinylpyrrolidone (PVP); polyethylene glycol (PEG), methyl cellulose, agarose, extracellular matrix proteins such as collagens, fibronectins, fibrin, glycosaminoglycans, and mixtures thereof. In general, 0.1 to 50% by weight of the composition of delivery polymer, is added to the insulin formulation to produce a gel.

Optional Adjuncts

Besides the insulin active, further adjuncts such as metal ions including zinc and chromium ions, anti-oxidants, antimicrobials, preservatives, opacifiers, colourants, perfumes, carrier proteins and buffers may be present in the pharmaceutical composition.

Product Preparation, Form, Use and Packaging

To prepare the pharmaceutical composition to be used in accordance with the present invention, the usual manner for preparing tissue treatment products may be employed. The active components are generally incorporated into a pharmaceutically acceptable vehicle in conventional manner. The active components can suitably first be dissolved or dispersed in a portion of the water or other solvent or liquid to be incorporated in the composition. The preferred compositions are water/saline solutions of insulin.

The compositions may be in the form of conventional tissue treatment products such as cream, gel, lotions

or solutions. The composition may be packaged in any suitable manner such as a jar, bottle, vial, tube or the like in a conventional manner.

5 The amount and frequency of application of the composition required for treatment will be readily apparent to one skilled in the art. In general, the treatment of a wound may be carried out by application, topically or by local injection, of a pharmaceutical
10 composition comprising the insulin to the wound one or more times daily. Typically, in solution or gel form, about 1ml of formulation is applied per cm² of the tissue trauma depending on the depth and severity of the wound to be treated. The extent of prevention
15 and/or reduction in scarring and enhanced re-epithelialisation of the tissue trauma will depend on the wound condition, the concentration of the active components, the amount of composition used and the frequency with which it is applied/injected.

20

In order that the present invention may be more readily understood, the following examples are given, by way of illustration only.

25

Example 1

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35

One of the most important cell types in both normal and pathological scar formation is the myofibroblast. These cells, which differentiate from the unwounded tissue cell type (fibroblasts), are responsible for laying down scar tissue. Indeed myofibroblasts remain present in hypertrophic scars up to four years after the original wounding event. An in vitro assay was accordingly developed to identify actives which prevent or reduce myofibroblast formation and thus identify actives which are effective in reducing and/or

preventing scar tissue formation.

Assay

5 . Fibroblast cultures were initiated from normal skin,
normal scars, hypertrophic scars (HTS) and burns scars.

Each culture was split into four parts (A to D) and
grown in the following different growth media:

10

(A) was grown in normal growth medium (hereinafter
referred to as NGM) which consisted of Dulbecco's
modified Eagles Medium (DMEM) plus 10% Foetal Calf
Serum (FCS);

15

(B) was grown in growth factor depleted medium
(hereinafter referred to as GF depleted) which is NGM
that is depleted of active polypeptide growth factors
including TGF β . It is prepared by treating FCS with a
reducing agent in order to attack and cleave the
20 disulphide bonds that determine the conformation and
thus the biological activity of the polypeptide growth
factors;

20

(C) (C₁-C₃) was grown in growth factor depleted medium
plus 10ng/ml-5 μ g/ml of insulin (hereinafter referred to
25 as GF depleted + I);

25

(D) (D₁-D₄) was grown in growth factor depleted medium
plus 1ng/ml-100ng/ml of insulin - like Growth Factor -
I) (IGF-I) (hereinafter referred to as GF depleted +
IGF-I).

30

The fibroblasts were grown in these media (which was
replenished twice weekly) for fourteen days, fixed and
stained immunohistochemically using an antibody
specific for α -smooth muscle actin (a marker of
35 myofibroblasts). The stain caused α -smooth muscle actin
to fluoresce green and accordingly cells that had

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stained green were identified as myofibroblasts. The number of these was counted, as was the total number of cells present, and the proportion of myofibroblasts was then calculated. In this manner, the number of
5 myofibroblasts present in each culture was determined as a percentage of the total number of cells in the culture population.

Results

TABLE 1

MEDIUM	Normal Skin	Normal Scar	HTS	Burns Scar
	NUMBER OF MYOFIBROBLASTS- PERCENTAGE OF TOTAL CELL POPULATION			
A - NGM	7.58	4.59	4.27	13.33
B - GF DEPLETED	19.15	34.71	35.14	42.55
C ₁ - GF DEPLETED + 10ng/ml INSULIN				16.44
C ₂ - GF DEPLETED + 100ng/ml INSULIN				15.91
C ₃ - GF DEPLETED + 5μg/ml INSULIN	5.26	6.14	3.23	8.20
D ₁ - GF DEPLETED + 1ng/ml IGF-I				40.79
D ₂ - GF DEPLETED + 10ng/ml IGF-I				43.90
D ₃ - GF DEPLETED + 50ng/ml IGF-I				51.51
D ₄ - GF DEPLETED + 100ng/ml IGF-I				47.00

Conclusions

A small but significant number of myofibroblasts are present in the NGM only cultures (see (A)). This phenomenon is thought to be due to the presence of TGFβ in the FCS which makes up the medium, which is known to

induce fibroblasts to differentiate into myofibroblasts whether they be derived for normal skin, normal scars or pathological scars.

5 A surprisingly large number of myofibroblasts are present in the GF depleted cultures (see (B)). It was expected that because TGF β was thought responsible for the presence of the myofibroblasts in NGM cultures, then the culture of fibroblasts in GF depleted medium
10 should reduce the number of myofibroblasts seen in the population. However, contrary to expectation, no matter what the source of fibroblasts, culture in GF depleted medium promoted fibroblast differentiation into myofibroblasts. This surprising result suggests that
15 FCS contains actives that inhibit myofibroblast formation, with the biological activity of those actives being strictly dependant on the presence of intact disulphide bonds.

20 During attempts to identify the polypeptide constituent of FCS that was responsible for inhibition of myofibroblast differentiation, it was discovered that insulin but not its related factor IGF-I was capable of inhibiting myofibroblast differentiation. (See C and D
25 of Table 1). Both growth factors were titrated over their respective biofunctional ranges (insulin: 10ng/ml-5 μ g/ml. IGF-I:1-100 ng/ml). Both of these factors are known to be present in serum and their biological activities are strictly dependant on the
30 presence of intact disulphide bonds.

Comparing B and C it can be seen that the addition of exogenous insulin specifically to fibroblasts cultured in GF depleted medium completely abrogates the
35 induction of myofibroblast differentiation seen in this medium. Importantly, and further surprisingly,

comparing the data in C & D for burns scar cells it can
be seen from the data presented in C that insulin is
equally effective at inhibiting myofibroblasts
differentiation for both normal skin, normal scar and
5 pathological cells and thus is suitable both for
preventing scar formation and reducing the extent of
scarring in both normal wound healing and pathological
scarring conditions. Further surprisingly the related
IGFs do not exhibit this technical effect thus
10 demonstrating that the effect is insulin specific.

This finding, that the single specific growth factor
insulin can prevent or at least reduce the formation of
myofibroblasts, has wide implications in the fields of
15 both cutaneous scarring and fibrosis generally. This
new use of insulin as a treatment for preventing and/or
reducing scar tissue formation is accordingly an
important medical advancement for tissue trauma
sufferers. This new use is particularly beneficial as
20 insulin also promotes the positive events of the wound
healing process such as enhancing the re-
epithelialisation of wounds and thus a dual action
treatment for wounds is accordingly provided for the
first time.

EXAMPLE 2

The following injectable composition was prepared in
~~conventional manner. It was found both to prevent and~~
30 reduce scar tissue formation when locally injection
into wounds in accordance with the present invention.
The formulation is also suitable for topical
application.

35 Each millilitre of the formulation contains:

100IU of insulin (either human insulin (pyr) which is
of recombinant origin produced in yeast), 3.78mg
dibasic sodium phosphate, 1.76mg m-Cresol, 0.715mg
phenol, zinc oxide, (content adjusted to provide 0.025
5 mg zinc ion), 0.28mg protamine sulphate, 16mg glycerin,
and water to dilute to the required concentrations.
The pH range of the composition is preferably 7.0-7.8,
but 10% sodium hydroxide or hydrochloric acid may be
used to adjust the pH, as required.

CLAIMS

1. Use of insulin for the manufacture of a medicament for reducing and/or preventing scarring.

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2. Use of insulin for the manufacture of a medicament for a dual action wound treatment comprising reducing and/or preventing scarring whilst accelerating and/or promoting wound healing.

10

3. Use according to claim 1 or 2 wherein the medicament is a topical composition or an injectable composition.

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4. Use according to claim 1, 2 or 3 wherein the insulin is present in an amount from 50 picograms (1.25×10^{-6} IU) to 1000 micrograms (25 IU) per millilitre of the composition.

20

5. Use of insulin substantially as hereinbefore described with reference to Example 1 and/or 2.



INVESTOR IN PEOPLE

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Examiner: J. P. Bellia
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Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:
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Other: ONLINE: EPODOC, WPI, JAPIO

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	EP 0 561 330 A1 (LIEDTKE PHARMED) See also WPI Abstract Accession No. 1993-296636 [05]	1-4
X	EP 0 162 007 A1 (GERIACO AG) See also WPI Abstract Accession No. 1985-291215 [47]	1-4
X	WO 96/25943 A1 (LIFE MEDICAL) See page 1 and pages 31-35	1-4
X	WO 96/02270 (GROPEP) See page 4 line 27 - page 5 line 23; page 6 line 26 - page 7 line 16	1-4
X	WO 93/10795 A1 (HINSON) See page 1 line 10-15 & Examples	1-4
X	US 5 591 709 (LINDENBAUM) See Example 4 and claim 1	1-4
X	WPI Abstract Accession No. 1998-589667 [50] & JP 100265405 A (NANBARA) See abstract	1-4
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X	WPI Abstract Accession No. 1989-071472 [10] & JP 010022254 A (TERUMO CORP) See abstract	1-4

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.